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GENOTOXIC DAMAGE *IN VIVO*, SUSCEPTIBILITY TO UVC AND X-RAYS, AND REPAIR EFFICIENCY *IN VITRO* LYMPHOCYTES FROM REFERENT AND OCCUPATIONAL EXPOSED TO MERCURY VAPOURS GROUP.

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ABSTRACT

For years mercury has been considered to be dangerous for human health. It was shown *in vitro* studies that mercury ions produce various types of DNA damage: single strand breaks as well as alkali labile lesions. The aim of this study was to compare levels of the DNA damage and cytogenetic damage induced *in vivo*, DNA's susceptibility to radiation, as well as repair capabilities of DNA damage induced *in vitro* by UV-exposure or X-rays, in lymphocytes from unexposed donors and from persons occupationally exposed to mercury vapours. In order to estimate cytogenetic damage, the analysis of sister chromatid exchange frequency (SCE) was used, while to detect DNA damage alkaline version of the single cell gel electrophoresis (SCGE) was applied. To analyse *in vitro* susceptibility of cells to genotoxic factors such as UV-C or X-rays, lymphocytes were exposed to 6 J/m^2 of UV or irradiated with 2 Gy of X-rays. After exposure the cells were incubated for 2 hours with or without the presence of phytohemagglutinin (agent stimulating cell divisions). In the present study, results do not show any statistically significant differences between the examined groups, either in the levels of DNA damage of untreated lymphocytes or in the sister chromatid exchanges. Neither the levels of DNA damage detected in lymphocytes after UV exposure and 2h incubation, without or in the presence of a cell- division stimulating agent, nor the repair efficiencies of the DNA damage induced by UV exposure, differed significantly between the unexposed group and that occupationally exposed to mercury vapours. Statistically significant higher levels of DNA damage measured in X-ray-irradiated lymphocytes, without incubation and after 2 hours of incubation, with or without the presence of phytohemagglutinin, were observed in the group exposed to mercury vapours. So, donors exposed to mercury vapours have shown a statistically lower repair efficiency of X-ray-induced DNA damage both in non-stimulated lymphocytes (70.8% for the exposed, 84.5% for the unexposed) and stimulated lymphocytes (85.7% for the exposed and 90.4% for the unexposed).

INTRODUCTION

For years mercuric compounds have been considered to be especially dangerous for man. It is known now that little concentrations of this element cause serious damage to the nervous system [1], immunity system [2], kidneys [3] and liver. They also constitute a serious threat to pregnant women, because they penetrate the blood – placenta barrier easily, thus damaging the embryo [4].

The aim of this study was a comparison of the molecular and cytogenetic damage detected in lymphocytes of unexposed donors and of people occupationally exposed to

mercury vapours. We were also interested to know whether occupational exposure to mercury vapours may affect individuals cellular susceptibility to radiation, as well as repair efficiency of DNA damage induced by UV-C and X-rays. In order to examine the cytogenetic damage, the sister chromatid exchange frequency was used [5], while to detect DNA damage, the alkaline version of the of the single cell gel electrophoresis (SCGE) also known as a comet method was applied [6, 7,8]. This method is a sensitive test conforming to the analysis of DNA damage induced by different chemicals and ionising radiation. Moreover, at high pH levels it permits one to detect various types of DNA damage such as single- and double-strand breaks, and DNA damage of alkali labile sites [9]. In order to analyse susceptibility to radiation and repair efficiency of DNA damage *in vitro,* two types of radiation (UV and X-rays) were used. These types of radiation constitute environmental exposure factors. The X-ray-radiation produces mainly single-strand breaks, much less abundant double-strand breaks, alkali labile sites and various types of oxidized purines and pyrimidines [10]. On the other hand, UV-C radiation causes pyrimidine dimmers, which could not be directly detected by the comet assay, but DNA strand breaks are produced in a excision process during incubation [11].

MATERIALS AND METHODS

Sampling and preparation of lymphocytes

Interviews were performed with a questionnaire considering health, life styles and habits, job conditions, and possibilities of hazardous exposure leading to increased genotoxic risk. The questionnaires followed the recommendation for a biological monitoring type of study [12]. Groups investigated consist of 25 donors occupationally exposed to mercury vapours (males, average age 37.8, length of occupational exposure 2-35 years) and 50 donors from the reference group (males, average age 38.5). Among the group of people occupationally exposed to mercury vapours 36% were smokers, with the 64% in referent group. In both investigated groups peripheral blood samples were collected into heparinized tubes and then were transported to the Laboratory of the Department of Radiation and Environmental Biology, Institute of Nuclear Physics, where in the part of the sample the cytogenetic culture procedure was performed as soon as possible. From the other part lymphocytes were isolated and frozen for further studies according to the standard procedure [6,7].

Culturing and screening for presence of sister chromatid exchanges (SCE)

To estimate cytogenetic damage induced *in vivo,* the sister chromatid exchange method was applied. All chemicals were used according to standard procedures [5,13]. Blood from a donor was incubated at 37°C using the RPMI 1640 medium supplemented with 20% fetal serum, BrdU and antibiotics. Lymphocytes were stimulated with phytohemagglutinin (PHA) or its Polish analogue LF-7. Cultures for sister chromatid exchanges were incubated for 72h. Two hours before the end of culturing, 0,1µl/ml colcemid solution was added to each culture. Fixation and staining were performed by standard cytogenetic methods [13]. The frequency of SCE were screened in the second mitosis. High frequency cells (HFC) were evaluated as percentage of cells displaying the number of exchanges per cell higher than 95% of population distribution in the reference group.

The proliferating rate index (PRI) was evaluated as reported in [14] as:

$$PRI=(M_1+2M_2+3M_3)/(M_1+M_2+M_3)$$

where $M_1+M_2+M_3$ were numbers of metaphases screened in the first, second and third mitosis respectively.

In vitro treatment

Frozen lymphocytes samples were defrosted 24h before the beginning of the experiment with the use of the standard procedure [6,7], and viability of cells (usually higher than 94%) was evaluated with an application of trypan blue technique. Lymphocytes then were resuspended in 5ml of cold PBS and divided into following study groups:

- No *in vitro* treatment for an evaluation of the damage induced *in vivo*,
- Exposure to UV-C (dose 6 J/m^2),
- Exposure to X-rays (dose 2Gy),
- Exposure to X-rays (dose 2Gy) and incubation for 2 hours at 37°C ,
- Exposure to X-rays (dose 2Gy) and incubation for 2 hours at 37°C in the presence of the factor stimulating lymphocyte divisions.

Exposures to UV-C and X-rays

To examine efficiency of the induction of the DNA damage and repair efficiency in donor's cells, two types of radiation producing various types of DNA damage were applied. For UV exposure, 60W quartz lamp (λ= 254nm) was used, and the exposure dose of 6J/m^2 was set by UVC-dosimeter (Ultra lum CDR-2). Exposure to UV was performed at room temperature. However, to avoid repair processes during the exposures, cells were exposed in a cold PBS on Petri dishes situated on the plate 0-4°C and then immediately divided into two parts for the following treatments:

- an incubation for 2hours at 37°C.
- an incubation for 2hours at 37°C in presence of the factor stimulating lymphocytes to divisions.

For X-ray irradiation a Philips machine model MCN 323 was used. Cells in eppendorf vials were settled in the polyethylene box containing an ice cubes in a water. Dosimetric measurements performed for those experimental conditions gave the dose rate 0.5Gy/min. After irradiation with the total dose of 2Gy samples were immediately transferred for farther procedures.

Analysis of the DNA damage by single cell gel electrophoresis (Comet) assay

In all groups of aliquots the alkaline version of the comet assay was applied in order to estimate the level of DNA damage. All chemicals were used according to standard procedures [6,7,11].

The suspension of lymphocytes sunken in agar LMA (95µl) was accumulated in layers on the microscopic slide with agar NMA (120µl). Then another layer of agar LMA (90µl) was added. These materials were left to gelation at 4°C. The slides were immersed in cold lysing solution (2,5M NaCl, 100mM EDTA, 1%, sodium sarcosinate, 100 mM TRIS) with 10% DMSO and 1% Triton X-100 added immediately before use. After lysis**,** the slides were transferred to the electrophoretic apparatus and immersed in freshly made, cold

electrophoretic buffer (1mM EDTA, 300mM NaOH) of pH=13. After 20 minutes of DNA unwinding, electrophoresis was carried out for 30min at 4°C (30V, 300mA). Then the slides were washed in 0,4 M buffer Tris of pH 7.5 (3 times for 5 minutes). All the stages, from lysis to the neutralisation of slides, were conducted in the dark in order to avoid any additional damage caused by light. After neutralisation, the slides were stained with 60µl of ethidinum bromide (17µg/ml), placed in a light-proof box and kept moist in PBS.

Detection of the DNA damage

After staining slides were analysed for the presence of DNA damage in the comets by means of the epifluorescence microscope Olympus BX-50 (100W mercuric lamp, excitation filter 515-560 nm, barrier filter from 590 nm) connected with the CCD camera. For the analysis of the DNA in the comets the Komet 3.0 program from the Kinetic Imaging Company (Liverpool, UK) was applied.

To estimate the DNA damage, three parameters from this software were used:

- **TL** - tail length (length of the comet tail),
- **tDNA**- tail DNA (DNA percentage in the comet tail),
- **TM** - tail moment (fraction of DNA in the tail multiplied by the tail length).

Statistics:

There were 75 cells from every slide analysed (25 from each of the three areas of a slide, 2 repetitions per dose). The statistical analysis was conducted by means of Student's t-test from Slide Write 3.0 software.

Results

Table 1 presents donors' data from the referent group such as age, smoking habits and DNA damage detected in defrosted lymphocytes and SCE frequency levels measured in the second division metaphases, and evaluated values of HFC and PRI. Table 2 shows similar donors' characteristics from occupationally exposed to mercury vapours group with length of exposure to mercury vapours, levels of similar biomarkers of genotoxic damage detected in untreated lymphocytes, as well as the statistical significance of these parameters between the reference and the exposed group. Outstanding results (i.e. those three standard deviations higher or lower than the mean value) are presented in a bold font. In general, no statistical differences can be noticed between the mean levels of damage neither molecular nor cytogenetic detected in untreated lymphocytes of referent and occupationally exposed groups.

Table 1.
Levels of DNA damage and SCE in lymphocytes isolated from referent donors.

Donors' code	Age	Smoking habits	Untreated lymphocytes					
			tDNA	TM	TL	SCE	%HFC	PRI
200	56	2	3.89	0.57	12.8	6.36	2.0	2.26
201	48	3	4.58	0.91	14.36	4.82	0.0	2.64
202	57	3	5.16	0.84	14.72	6.70	3.0	2.27
203	23	1	3.1	0.49	11.47	5.54	1.0	2.58
204	30	2	4.73	1.28	19.22	7.53	9.0	2.37
205	45	2	7.29	0.96	10.92	7.94	8.0	2.24
206	46	2	20.05	2.1	10.57	8.77	14.0	2.34
207	24	2	4.33	0.46	8.95	8.78	14.0	2.49
208	41	1	3.49	0.56	12.2	7.10	4.0	2.25
209	47	3	3.97	0.72	14.4	7.94	8.0	2.12
210	24	1	4.73	0.67	11.98	8.45	7.0	2.34
211	34	3	6.08	0.86	10.23	6.68	5.0	2.39
212	24	3	5.79	0.85	12.17	6.30	3.0	2.67
213	33	2	6.69	1.02	13.23	8.85	14.0	2.48
214	21	2	5.7	0.99	15.38	7.79	6.0	2.58
215	32	2	5.63	0.84	14.49	9.27	19.0	2.34
216	66	2	5.05	0.91	16.62	9.60	18.0	**1.98**
217	43	2	4.1	0.36	7.26	7.10	8.0	2.37
218	55	2	4.60	0.54	9.24	5.38	0.0	2.54
219	42	1	5.15	1.17	15.86	4.72	0.0	2.63
220	47	2	4.54	0.76	13.96	5.40	0.0	2.48
221	40	2	4.56	0.48	9.28	7.26	5.0	2.54
222	36	1	5.42	0.78	10.84	5.25	1.0	2.36
223	59	2	4	0.96	16.59	6.32	5.3	2.16
224	30	2	4.47	0.51	7.92	6.64	2.0	2.56
225	33	2	5.77	1.09	14.71	7.59	3.0	2.54
226	23	1	4.09	0.61	13.31	5.87	0.0	2.68
227	50	2	4.31	0.6	11.93	5.86	0.0	2.46
228	20	1	5.66	0.74	10.09	6.30	2.0	2.61
229	36	2	4.29	0.9	18.39	8.18	11.0	2.27
230	23	1	4.48	0.54	9.98	5.93	1.0	2.71
231	23	2	3.08	0.56	16.65	6.35	1.0	2.64
232	37	2	3.94	0.41	9.35	8.96	19.0	2.48
233	35	2	4.01	0.51	10.93	8.02	10.0	2.58
234	37	1	3.66	0.5	12.33	6.31	1.0	2.49
235	34	1	3.57	0.47	9.12	6.51	2.0	2.46
236	39	2	4.91	0.71	10.85	9.25	19.0	2.52
237	38	2	5.29	0.73	12.24	**10.86**	**34.0**	2.27
238	34	1	4.74	0.56	10.52	6.97	5.0	2.50
239	32	2	3.78	0.81	16.5	8.51	11.0	2.38
240	38	1	4.54	0.51	9.58	6.60	1.0	2.30
241	34	2	4.95	0.87	12.52	8.52	6.0	2.50
242	50	2	3.80	0.42	8.76	8.10	14.0	2.18
243	47	2	6.16	0.98	13.25	6.35	4.0	2.40
244	47	1	4.65	0.66	12.02	7.00	1.0	2.47
245	49	1	3.28	0.34	8.87	7.25	2.0	2.33
246	36	2	5.39	0.77	10.84	6.13	0.0	2.56
248	44	1	3.96	0.60	11.41	5.72	0.0	2.70
249	44	1	3.93	0.45	9.89	6.60	3.0	2.58
Mean	38.5		5.00	0.73	12.22	7.15	6.25	2.44
±SD			±2.4	±0.3	±2.8	±1.4	±7.0	±0.2

1-non-smoker, 2-smoker, 3-ex-smoker, SCE-sister chromatid exchanges in the second metaphase, HFC- percent of cells displaying number of exchanges per cell higher than 95% of population distribution in the reference group, PRI- proliferating rate index.

Table 2.
Levels of DNA damage and SCE in lymphocytes isolated from donors occupationally exposed to mercury vapours.

Donors' code	Exposure length	Age	Smoking habits	Untreated lymphocytes					
				tDNA	TM	TL	SCE	%HFC	PRI
1	17	48	1	5.07	0.84	11.06	6.09	2.0	2.65
2	32	52	2	5.45	0.82	10.34	6.04	3.0	2.62
3	28	53	2	3.64	0.38	6.44	7.46	8.0	2.47
4	8	31	1	5.82	1.30	14.97	6.89	6.0	2.43
5	20	46	2	4.92	0.47	7.16	**10.14**	**28.0**	2.42
6	15	37	2	5.53	0.74	12.69	7.14	6.0	2.19
7	2	40	1	4.37	0.45	7.93	6.54	4.0	2.56
8	3	28	1	5.34	0.87	10.88	7.76	10.0	2.18
9	8	33	1	4.37	0.69	8.92	7.36	2.0	2.39
10	2	27	2	3.91	0.50	8.82	7.81	10.0	2.43
11	8	44	1	4.76	0.50	9.18	6.26	2.0	2.66
12	25	42	1	5.50	1.15	13.88	6.88	12.0	2.43
13	11	36	1	3.02	0.35	5.97	6.20	4.0	2.39
14	11	28	1	5.68	1.01	12.91	8.44	12.0	2.51
15	4	40	1	3.44	0.33	7.15	7.44	6.0	2.60
16	26	43	2	4.15	0.53	9.00	5.92	6.0	2.30
17	29	50	1	3.97	0.39	7.61	8.02	12.0	2.41
18	29	46	2	4.39	0.49	9.32	9.60	16.0	2.47
19	35	53	2	3.71	0.55	8.46	7.10	6.0	2.48
20	2	22	2	3.67	0.39	8.19	6.16	5.0	2.53
21	5	26	1	4.34	0.49	7.54	9.02	16.0	2.61
22	17	44	1	4.52	0.52	7.76	8.16	12.0	2.35
23	4	24	1	4.55	0.58	9.17	7.78	6.0	2.50
24	6	27	1	5.40	0.84	14.55	8.87	15.0	2.55
25	3	25	1	5.46	**1.45**	12.90	7.14	10.0	2.66
Mean		37.8		4.61	0.67	9.74	7.49	8.76	2.47
±SD				±0.8	±0.3	±2.6	±1.1	±5.9	±0.1
P<				ns	ns	ns	ns	ns	ns

1-non-smoker, 2-smoker, 3-ex-smoker, SCE-sister chromatid exchanges in the second metaphase, HFC- percent of cells displaying number of exchanges per cell higher than 95% of population distribution in the reference group, PRI- proliferating rate index.

Table 3 shows results of the analysis of DNA damage after exposures of lymphocytes of people from the referent group to X-rays or UV-C on. These results were expressed with the help of average values of parameters such as tail DNA (tDNA) and tail length (TL). The first column presents donors' codes. The next two double columns show the mean values of parameters measured after 6J/m^2 of UV and 2-hour incubation at 37°C, in the presence or absence of PHA respectively. The last three double columns present the average values of parameters measured in cells right after X-ray-irradiation (2Gy) and after 2-hour incubation at 37°C, in the presence or absence of the phytohemagglutinin respectively.

Table 3.

Average parameter values; tail DNA (tDNA) and tail length (TL) measured in lymphocytes of unexposed people. Lymphocytes exposed to X-ray radiation (dose 2Gy) or UV exposure (dose 6J/ m^2) : without incubation and after 2h incubation at 37°C, without and with PHA presence.

Donors' code	UV (6J/ m^2)				X-rays (2Gy)					
	2h incubation without		2h incubation with PHA		Without incubation		2h incubation without		2h incubation with	
201	63.2	72.3	51.6	60.4	18.7	56.1	4.4	27.5	nd	
202	60.9	81.1	51.6	64.6	18.1	59.8	4.3	23.0	nd	
203	63.4	74.9	58.0	76.0	15.2	55.2	5.4	11.3	4.7	13.6
204	41.2	56.0	**29.5**	**42.4**	15.8	55.6	4.2	21.9	nd	
205	50.1	70.8	36.3	68.5	26.8	32.4	4.6	11.3	5.7	16.7
206	62.3	74.7	41.2	58.0	15.5	46.6	5.1	14.5	7.0	14.1
207	45.4	90.3	40.2	62.8	13.7	74.6	3.9	10.6	3.8	10.0
208	63.1	85.2	45.6	61.2	15.0	57.2	5.1	15.7	4.8	11.1
209	55.7	**99.5**	47.2	73.7	19.1	65.3	4.1	22.9	nd	
210	65.6	76.9	40.5	56.0	16.0	54.0	11.5	20.1	5.5	12.2
211	51.9	70.7	46.8	57.7	19.3	25.7	6.4	11.6	6.1	9.6
212	50.3	74.7	42.7	65.1	22.7	46.3	7.3	26.0	6.6	16.0
213	52.8	77.3	39.6	57.5	25.6	50.0	7.7	18.2	7.8	20.5
214	56.9	64.5	40.3	67.1	24.6	39.1	6.3	18.0	6.9	18.3
215	55.2	72.2	47.9	53.6	20.6	42.0	7.6	16.6	5.7	**22.4**
216	47.9	69.9	40.7	61.2	20.4	45.4	5.3	12.8	6.0	11.2
217	48.8	68.7	41.3	61.5	22.8	44.7	6.7	14.3	6.8	**23.4**
218	7.1	74.8	41.2	52.7	15.1	56.3	6.1	9.7	4,1	6,3
219	59.9	80.7	49.2	68.4	14.6	54.6	6.8	13.7	4.4	13.8
220	48.9	66.4	38.5	57.5	14.2	72.9	4.0	21.2	nd	
221	55.0	72.5	35.9	49.3	12.2	64.9	5.1	18.7	nd	
222	38.2	59.9	30.5	55.3	24.7	48.8	8.4	23.0	8.1	18.9
223	69.2	85.0	**66.0**	72.9	14.8	56.6	8.4	22.1	5.8	21.1
224	57.0	**96.2**	41.3	56.2	20.9	51.6	4.5	10.5	4.1	8.7
225	61.4	80.8	54.6	71.3	10.3	69.8	6.2	19.3		
226	63.3	88.1	59.8	76.2	11.4	64.3	5.0	22.4		
227	53.7	84.3	46.4	71.6	13.7	63.9	4.4	18.9		
228	57.4	71.0	45.0	47.8	20.6	50.3	7.0	16.2	5.9	14.4
229	53.5	72.2	49.4	49.4	26.2	41.8	7.5	17.2	6.6	12.7
230	59.9	80.9	52.1	73.9	19.8	57.2	4.8	21.7		
231	57.6	79.2	44.2	67.2	13.5	58.2	5.5	27.8		
232	55.2	67.7	50.3	51.4	17.8	53.9	4.4	9.9	6.2	12.7
233	56.3	68.0	54.2	58.0	21.1	45.8	14.3	12.5	**13.1**	12.0
234	49.6	66.2	44.5	56.4	28.7	40.8	4.6	13.6	3.6	14.0
235	42.0	58.3	38.6	51.0	30.4	41.3	5.7	17.9	4.8	9.3
236	62.2	93.5	44.0	54.6	16.9	58.3	4.6	20.3		
237	62.7	87.8	48.7	66.3	15.4	45.0	8.2	22.4	5.9	12.0
238	52.1	68.0	38.0	53.6	19.6	36.6	6.2	14.6	5.4	11.7
239	52.2	67.6	42.1	52.4	13.5	58.7	5.8	23.9		
240	50.8	78.3	36.2	52.7	18.1	49.2	6.7	15.3	4.6	8.9
241	64.8	90.1	49.2	69.6	20.2	50.1	7.6	21.7	5.3	10.8
242	56.3	77.6	40.3	61.3	17.2	50.2	5.3	13.0	5.1	10.5
243	53.4	81.0	35.8	55.4	17.2	47.4	5.0	16.6	4.6	9.8
244	57.0	79.0	40.1	57.0	19.2	49.8	5.0	12.2	4.9	9.6
245	61.6	78.4	59.9	54.0	15.9	61.7	6.6	15.2	5.4	12.5
246	54.0	83.9	33.5	60.1	18.3	56.0	5.5	14.2	6.1	12.6
248	61.6	72.4	51.2	62.2	17.7	53.8	4.9	24.5		
249	64.3	75.7	51.9	67.3	18.9	64.7	8.5	17.7	6.8	13.9
Mean	56.0	76.6	45.0	60.4	18.5	52.8	6.0	17.7	5.6	13.8
±SD	±9.6	±9.4	±7.7	±8.0	±4.4	±10.0	±2.0	±4.9	± 1.7	±4.1

n d - no data

Table 4 shows a comparison of the mean values of tail DNA and tail length parameters obtained for lymphocytes from exposed donors after the same treatments as for referent group, along with the statistical significance of these parameters for the referent and the exposed group.

Table 4.
Average parameter values; tail DNA and tail length measured in lymphocytes of people occupationally exposed to mercury vapors. Lymphocytes exposed to X-ray radiation (dose 2Gy) or UV exposure (dose 6J/ m^2) : without incubation and after 2h incubation at 37°C, without and with PHA presence.

Donors' code	UV (6J/ m^2)				X-rays (2Gy)					
	2h incubation without PHA		2h incubation with PHA		Without incubation		2h incubation without PHA		2h incubation with PHA	
	tDNA	TL	tDNA	TL	tDNA	TL	tDNA	TL	tDNA	TL
1	45.8	71.7	27.1	39.4	25.1	66.6	**16.7**	39.9	9.9	29.2
2	**35.9**	**52.8**	**22.7**	**36.8**	20.7	59.9	12.2	28.6	8.2	26.2
3	53.5	85.5	40.3	59.2	20.8	71.0	13.2	39.7	9.9	29.9
4	49.8	66.9	41.6	59.2	20.3	73.1	11.0	40.3	10.4	37.3
5	47.2	73.8	39.5	58.5	16.5	56.0	4.9	11.7	3.3	7.9
6	48.5	81.0	39.2	64.8	19.1	58.6	5.8	12.5	4.9	11.9
7	47.8	79.2	35.7	66.4	15.3	54.3	4.6	14.5	2.8	9.0
8	58.5	78.1	41.8	56.4	19.9	54.2	7.4	15.1	4.9	10.5
9	49.8	71.2	37.7	61.4	16.4	57.2	5.2	10.4	4.1	8.4
10	41.3	58.2	32.4	54.4	14.2	58.7	9.7	31.6	8.2	24.0
11	50.2	80.7	42.2	68.6	15.9	51.3	5.0	11.2	3.8	7.8
12	57.1	90.3	42.8	65.1	18.7	70.0	11.0	36.8	8.9	30.3
13	39.3	71.7	38.5	62.6	10.8	34.2	8.9	28.8	7.8	23.9
14	49.5	85.9	41.5	66.0	15.1	45.7	6.6	24.1	6.1	23.3
15	45.4	77.0	31.6	60.0	13.5	68.4	4.7	10.0	3.9	8.1
16	48.8	66.1	34.5	51.0	nd	nd	nd	nd	nd	nd
17	49.7	83.3	42.0	68.5	nd	nd	nd	nd	nd	nd
18	51.9	87.5	45.3	70.5	nd	nd	nd	nd	nd	nd
19	50.9	87.4	37.1	58.8	nd	nd	nd	nd	nd	nd
20	44.9	62.5	39.9	58.3	15.4	55.0	9.0	30.9	7.4	24.2
21	42.0	61.4	29.6	44.6	19.1	51.8	10.1	28.5	7.8	25.0
22	43.7	68.3	32.6	48.5	18.1	47.0	11.4	33.4	8.1	26.3
23	57.4	79.7	38.3	69.4	18.7	60.3	8.3	19.8	6.8	8.8
24	**62.8**	93.6	44.9	78.0	16.2	63.8	7.7	18.8	4.9	7.8
25	47.1	70.4	37.5	60.6	18.1	68.3	7.6	15.2	4.5	7.7
Mean	48.7	75.4	37.4	59.5	17.5	58,4	8.6	23.9	6.5	18.5
±SD	±6.1	±10.6	±5.6	±9.7	±3.1	±9.6	±3.2	±10.7	±2.4	±9.9
P<	ns	ns	ns	ns	ns	0.05	0.01	0.01	ns	0.05

nd - no data

After UV-C treatment none of the parameters presented in the Tables 3 and 4 differed significantly. All treatments with X-rays exposure induced higher damage in lymphocytes of donors from occupationally exposed group than in lymphocytes of donors from referent group. Length of the comet observed right after irradiation, percent of DNA in the comet tail and comet length after incubation of irradiated lymphocytes, and also comet's length in stimulated to division irradiated lymphocytes were statistically significantly higher in lymphocytes from exposed group than from referent group.

The mean values of the third parameter (tail moment) determining the level of DNA damage for lymphocytes of the referent group after UVC-exposure and incubation for 2 hours at 37°C in the presence or absence of PHA, are presented in Figures 1. The respective values detected in lymphocytes of donors from occupationally exposed group are presented on Figure 2. Figures 3 and 4 show mean values of tail moment, for lymphocytes from referent and exposed group respectively, measured *in vivo* and after various X-rays treatments.

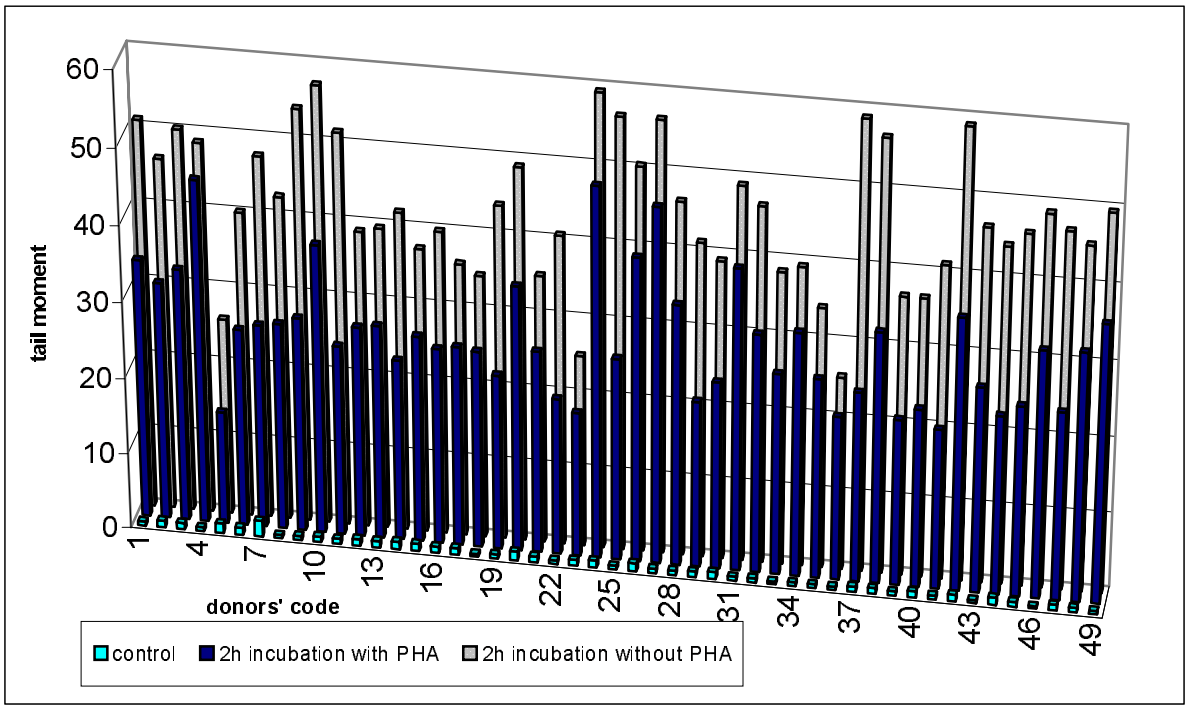

Figure 1. Mean values of tail moment in lymphocytes of unexposed donors: defrosted, after 6J/m^2 of UV exposure and after 2h incubation, with or without PHA.

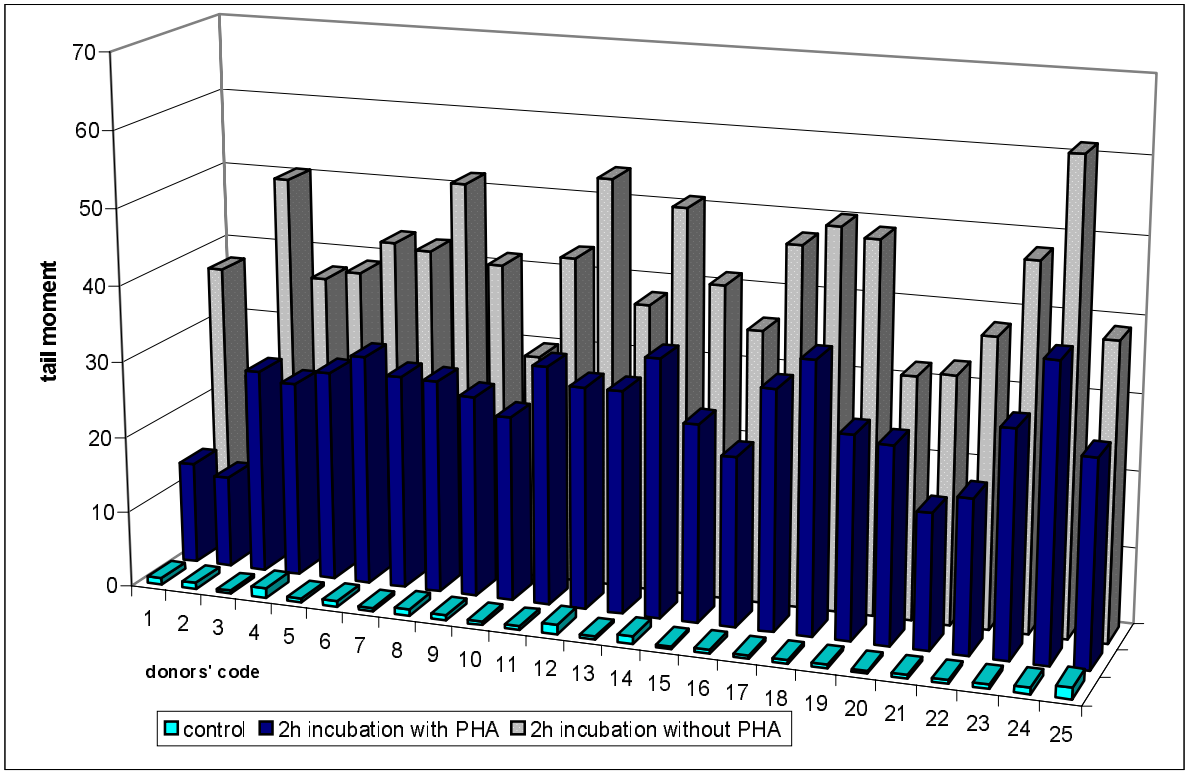

Figure 2. Mean values of tail moment calculated for lymphocytes of donors exposed to mercury: defrosted, after 6J/m^2 of UV exposure and after 2h incubation, with or without LF-7.

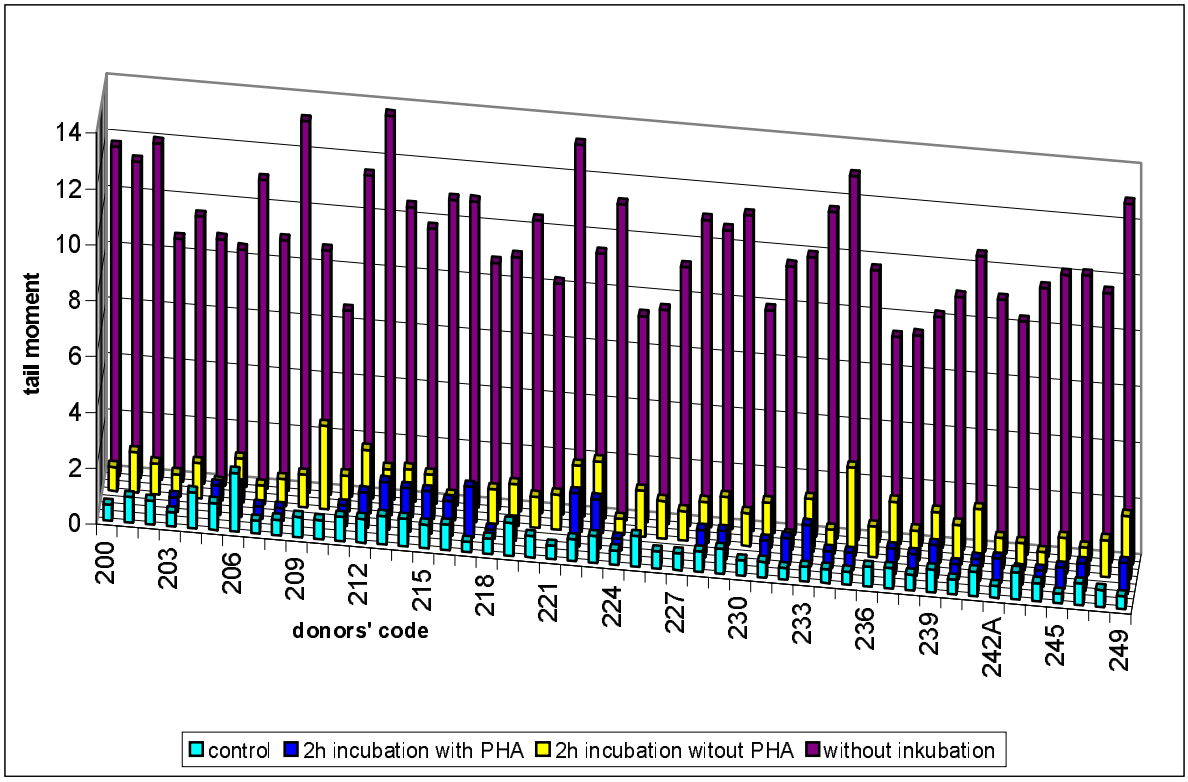

Figure3. Mean values of tail moment in lymphocytes of unexposed donors: defrosted, after 2Gy of X-rays without incubation and after 2h incubation, with and without PHA.

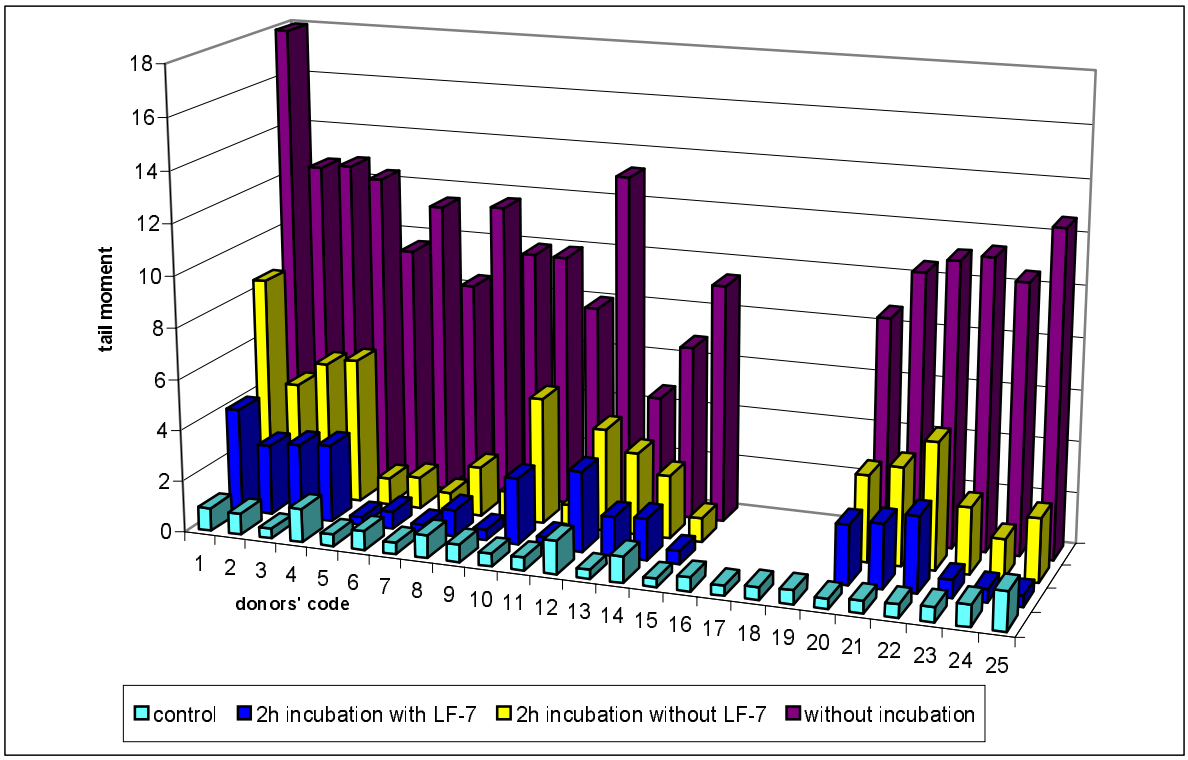

Figure4. Mean values of tail moment calculated for lymphocytes of donors exposed to mercury: defrosted, after 2Gy of X-rays without incubation and after 2h incubation, with and without LF-7.(nd - no data)

Table 5 presents results of statistical analysis of this parameter for compared groups.

Table 5.
Comparison of mean values of tail moment parameter observed in lymphocytes of unexposed donors vs those exposed to mercury vapours.

Group	Referent	Exposed	Significance
untreated	0.73±0.3	0.67±0.3	ns
UV(2h)	44.5±8.5	42.1±8.6	ns
UV(2h)+PHA	29.0±7.0	26.7±6.1	ns
X(0)	9.98±1.7	10.6±2.7	Ns
X(2h)	**1.4±0.6**	**3.1±2.0**	0.01
X(2h)+PHA	**1.0±0.4**	**1.7±1.2**	0.01

UV(2h) – UV-exposed and 2-hour-incubated lymphocytes, UV*(2h)+ PHA*- UV-exposed and 2-hour-incubated in presence PHA lymphocytes, *X(())* - X-ray-irradiated lymphocytes, *X(2h)* - X-ray-irradiated and 2-hour-incubated lymphocytes, *X(2h)+ PHA*- X-ray-irradiated and 2-hour-incubated in presence PHA lymphocytes

Again, no significant difference between referent and occupationally exposed group is observed after UV treatment. And again, after X-ray-exposure results obtained for people exposed to mercury vapours are higher that from referent group, and levels of residual damage detected after two hours of incubation, both without and with stimulation to divisions, are significantly higher in lymphocytes of persons exposed to mercury than in those from referent group.

In order to enhance the kinetics of excision process lymphocytes were stimulated into divisions by incubation in presence of cellular mitogen (PHA). Comparison of DNA damage measures performed in stimulated and no stimulated lymphocytes is shown in table 6.

Table. 6
Influence of the presence of the PHA stimulator on DNA damage in lymphocytes during 2-hour-incubation.

Group	2h incubation	UV	X -rays
		TM±SD	TM±SD
RG	-	44.5±8.5	1.4±0.6
RG	PHA	29.0±7.0	1.0±0.4
P<		0.01	0.01
Exp	-	42.1±8.6	3.1±2.0
Exp	PHA	26.7±6.1	1.7±1.2
P<		0.01	0.01

As is seen from the table 6 the stimulation of lymphocytes to divisions have significantly lowered the value of the tail moment in all investigated groups. In order to appreciate of the influence occupational exposure to mercury vapours on the efficiency of excision process and enhancement of repair kinetics of the DNA damage produced by these types of radiation, following parameters were calculated according to the formula below:

RR_{UV} =[(TM_{UVPHA} -$TM_{UV(2h)}$)/ $TM_{UV(2h)}$]x 100

where:

RR_{UV} – enhancement in repair rate efficiency of UV induced DNA damage in lymphocytes, $TM_{UV(2h)}$ and $TM_{UV(PHA)}$ were comet tail moments detected in lymphocytes exposed to UV and incubated for two hours in the absence or presence of PHA respectively.

Table 7 presents the mean values of percentages of enhancement of repair process due to stimulation of exposed cells to divisions observed in lymphocytes from the unexposed and exposed groups. The comparison of the obtained results shows a similar level of repair efficiency of UV-induced DNA damage in lymphocytes from the referent and exposed groups.

To appreciate the influence of the occupational exposure to mercury vapors on the susceptibility of the cells to the induction of the DNA damage by ionising radiation, and the efficiency of the repair of the damage induced, the following parameters were calculated from the measures obtained:

- Susceptibility to X-rays
  $Sx=TM_{X0}-TM_0$
  where: TM_{X0} was comet tail moment detected in lymphocytes right after X-ray-irradiation, and TM_0 was comet tail moment detected in untreated lymphocytes.
- Repair rate efficiency of X-ray-induced damage in lymphocytes *(RE)*; $RRE=TM_{x(0)-}TM_{(2h)}/TM_{(x0),}$ where: TM_{X0} was comet tail moment detected in lymphocytes right after X-ray-radiation, and $TM_{(2h)}$ was comet tail moment detected in X-ray-irradiated and 2-hour- incubated lymphocytes.
- Repair efficiency of X-ray-induced damage in stimulated lymphocytes *(REs)*; $REs=TM_{x(0)-}TM_{(PHA)}/TM_{(x0),}$ where: TM_{X0} was comet tail moment detected in lymphocytes right after X-ray- radiation and $TM_{(PHA)}$ was comet tail moment detected in X-ray-irradiated and 2-hour-incubated lymphocytes in the presence of PHA

The mean values of the results obtained are shown in the table 7. The second column shows the mean values of susceptibility to X-rays calculated according to the presented above formula. The third and fourth columns show respectively the mean values of DNA damage repair efficiencies evaluated for lymphocytes no stimulated or stimulated to divisions.

Table.7

Comparison of mean values of UV induced damage repair rate and susceptibility to radiation and repair efficiency in X-ray-irradiated lymphocytes of persons from referent group vs group exposed to mercury vapours.

Group	RR_{UV}	S_X	RRE	RE_S
RG	34.1	9.2	85.7%	90.3%
Exp	36.3	9.9	70.8%	84.5%
P>	ns	ns	0.01	0.01

Statistical analysis of results presented in the table 7 shows although slightly higher, but not significantly, susceptibility to ionizing radiation of lymphocytes from exposed group. Results show also a significantly lower repair efficiency of DNA damage induced by ionizing radiation in lymphocytes from the donors occupationally exposed to mercury vapors, for both cases cells stimulated and no stimulated into divisions.

To investigate whether there is a positive association between the length of exposure and the susceptibility to X-rays induced DNA damage, or repair capacity of the lymphocytes of people occupationally exposed to mercury vapours, we have divided donors into three

groups depending on the number of their years of work. Mean values of the level of DNA damage and susceptibilities and repair efficiencies evaluated for the groups categorized according to years of work are presented in table 8.

Table. 8
Influence of length of donors' exposure to mercury vapours on DNA damage (evaluated for tail moment) in their lymphocytes.

years of work	0-10	11-20	21-35
	TM	TM	TM
X(2h)	2.49	3.57	4.63
X(2h)+LF-7	1.23	1.85	2.92
RE	75.68%	65.14%	64.14%
Res	88.00%	81.93%	77.38%

X(2h) - X-ray-irradiated and 2-hour-incubated lymphocytes, *X(2h)+ LF-7*- X-ray-irradiated and 2-hour-incubated in presence LF-7 lymphocytes, *RE* – repair efficiency X-ray-irradiated and 2-hour-incubated lymphocytes, *REs* – repair efficiency X-ray-irradiated and 2-hour-incubated in presence LF-7 lymphocytes

There is clearly seen tendency of length of work influence on the biomarkers investigated. We have also checked whether smoking habit might affects our results. As is shown in the Table 9, none of the biomarkers under the studies have shown influence of smoking.

Table 9.
Comparison of mean values of tail moment lymphocytes between non-smokers and smokers donors in exposed to mercury vapours group.

Group	Exposed		
	Non-smokers	Smokers	Significance
	TM	TM	
untreated	0.74	0.54	ns
SCE	7.38	7.49	ns
UV(2h)	43.51	40.96	ns
UV(2h)+LF-7	26.67	26.15	ns
X(0)	10.59	11.57	ns
X(2h)	2.88	3.67	ns
X(2h)+LF-7	1.50	2.02	ns
RRuv	36.23%	36.00%	ns
RE	71.49%	67.26%	ns
REs	85.15%	81.98%	ns

UV(2h) – UV-exposed and 2-hour-incubated lymphocytes, UV*(2h)+ LF-7*- UV-exposed and 2-hour-incubated in presence LF-7 lymphocytes, *RRuv* – repair rate efficiency UV-exposed lymphocytes, *X(())* - X-ray-irradiated lymphocytes, *X(2h)* - X-ray-irradiated and 2-hour-incubated lymphocytes, *X(2h)+ LF-7*- X-ray-irradiated and 2-hour-incubated in presence LF-7 lymphocytes, *RE* – repair efficiency X-ray-irradiated and 2-hour-incubated lymphocytes, *REs* – repair efficiency X-ray-irradiated and 2-hour-incubated in presence LF-7 lymphocytes

Discussion

Thanks to the research carried out since the beginning of the 80s [15,16,17], it has been found that mercuric compounds have a mutagenic effect. *In vitro* studies have shown that this chemical element induces single DNA-strand breaks and alkali-labile sites [9]. Numerous, papers report that mercury compounds may cause also an enzymatic alterations [18] and induce a cytogenetic damage such as anomalies in the quantity of chromosomes [19], increase in chromosomal aberrations [20] and the quantity of micronuclei [21]. The aim of our research was to investigate whether occupational exposure to mercury vapors can affect the molecular and cytogenetic damage detected in lymphocytes of exposed people or biomarkers of susceptibilities and repair efficiency evaluated from their lymphocyte responses in studies *in vitro.*

The statistical analysis conducted by means of Student's t-test did not show any statistically significant differences in the measured parameters (tail moment, tail DNA, tail length) determining the level of DNA damage in defrosted lymphocytes between the referent and the exposed groups (Table.2). Although, the level of SCE and HFC in the group exposed to mercury was higher (Table.2), though statistical analysis of cytogenetic damage (frequency sister chromatid exchanges) induced *in vivo*, as well as mean values of HFC, and PRI also did not show any significant differences between investigated groups. Similar results were obtained by Popescu and al. [22], where people occupationally exposed to mercury compounds showed no statistically significant differences in SCE frequency, however, they showed a statistically significant increase in chromosomal aberrations. Research conducted *in vitro* by Wulf and al. [23] shows linear correlation between Hg concentration in blood and SCE. However, in his study Hg blood concentration oscillated in the 23.15 to 65.41ul/l range, and unfortunately we can not compare this directly with our data, where the concentrations in the blood was not available.

Statistical analysis performed for two groups of UV-exposed lymphocytes (one incubated for 2h at 37°C in the presence, another in the absence of PHA stimulator), have not indicated any statistically significant influence of exposure either on the level of DNA damage induced (Table 4), nor on the repair efficiency (Table 8). Then, our results suggest that occupational exposure to mercury vapours has no modifying influence on DNA's susceptibility to UV or the repair efficiency of DNA damage induced by this radiation. These results are in agreement with the data obtained by Christie et al. [24], who showed that mercury does not interfere with UV-induced damage repair.

In the present study we investigated whether exposure to mercury vapours influences the increase of DNA damage produced by X-rays. In order to examine the susceptibility of cells to ionising radiation, the level of DNA damage right after exposure and repair efficiency of DNA damage was determined by measuring residual damage after 2-hour-incubation, without and with presence of PHA. The statistical analysis conducted for X-ray-irradiated lymphocytes from exposed and referent groups immediately after exposure show statistically significant differences in tail length parameter ($p=0.05$). Similarly, the comparison of the levels of DNA damage in irradiated and 2-hour- incubated lymphocytes without the presence of phytohemagglutinin show statistically significant differences between the unexposed and exposed groups in the tail moment ($p=0.01$) and the tail length ($p=0.01$) parameters (Table.4, 5). Again, a visible effect of mercury vapours was observed in lymphocytes irradiated with X-rays and incubated in the presence of PHA. The statistical significance in the level of DNA damage between the reference group and the group occupationally mercury exposed was found for the tail moment ($p=0.05$), and for the tail length ($p=0.01$) parameters (Table.4, 5).

However, comparison of cells susceptibility to radiation, evaluated on the base of described above formula, have indicated no significant differences in the investigated groups (Table.7). An analysis of the repair rate efficiency of the X-rays induced DNA damage shows a statistically significant (p=0.01) decrease in this parameter in the groups exposed to mercury vapours, both in stimulated cells (85.7%) and in non-stimulated ones (70.8%), in comparison to the reference group (90.4% and 84.5% respectively). Our data are supported by the results published by Cantoni et al. [25], who showed that mercury inhibits the repair of the X-ray-induced DNA damage by inhibiting polymerase.

The examination also confirms the influence of the factor stimulating lymphocytes divisions (PHA) on the level of DNA damage during 2-hour-incubation. The statistical analysis showed significant differences (p=0.01) between lymphocytes incubated for 2h, in the presence and in the absence of PHA stimulator after X-ray- and UVC-exposure. These findings correspond with the results obtained by Visvardis et al. [26], who suggested that the factor stimulating lymphocyte divisions (LF-7) activates enzymes participating in the DNA damage repair processes.

In order to investigate the influence of donors' length of exposure to mercury vapours on the level of the resulting DNA damage and repair efficiencies, the statistical analysis was conducted (Table.8). The analysis showed an increase of X-ray-induced DNA damage level in lymphocytes incubated in the presence or absence of PHA, with increase in the years of exposure. Also, the X-ray-induced DNA damage repair rate efficiency decreases with increasing years of exposure. Moreover, we compared the level of DNA damage estimated in the lymphocytes of smokers and non-smokers (Table 9). The analysis of our results did not show any affecting of our results of the level of DNA damage by smoking habits.

In conclusion, our results show, that occupational exposure to mercury vapours leads to an increase in X-ray-induced DNA damage and to a decrease in their repair efficiency.

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